Supporting Information

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SI Text

Caged Laboratory Experiments. Caged experiments using free-flying mosquitoes were conducted to develop an effective design for the pyriproxyfen-treated dissemination stations to be used in the field. Before this caged work, all concept proofs of using adult mosquitoes to carry JHAs to aquatic habitats had been carried out in highly artificial laboratory conditions (1). These caged experiments were also designed to demonstrate the relationship between the repeated contaminations of aquatic habitats, the consequent increase in pyriproxyfen dose, and the resultant increasing mortality of the juveniles therein.

A wooden framed, gauze cage, 2 m × 2 m was built indoors at the entomology facility in the Laboratorio de Salud Publica, Iquitos, Peru (27 ± 3 °C). Three experiments, each comprising 3 replicates, were carried out by using 2 designs of pyriproxyfen-treated dissemination stations and 2 densities of female mosquitoes (Fig. S1). The dissemination station designs consisted of a rolled sheet or a pot. The former consisted of a 0.36-m^2 sheet of black plastic painted with vegetable oil and dusted with the equivalent of 5 g pyriproxyfen/m^2 pulverized to the consistency of talcum powder (equivalent to 0.025 g a.i per m^2). This was rolled into a tube (≈20 cm in diameter) and suspended from the midpoint of the cage. The impact of this design was tested in combination with 500 or 50 female mosquitoes. The pot design consisted of a 1-L plastic disposable tub lined with black cloth treated with pyriproxyfen at the same rate as above. The tub held 200 mL of water to dampen the cloth and keep the pyriproxyfen dust in place. This pot design was placed in the center of the cage. It was tested with 50 females only (as we had established for the rolled sheet design that the use of 500 females was unnecessary to affect transfer of the JHA).

In each replicate, for both designs (rolled sheet and pot), a single dissemination station was placed inside the cage. Six oviposition sites (1-L disposable containers, lined with paper toweling, containing 200 mL of tap water and a small amount of fish food) were placed on the floor of the cage. Each of these was seeded with 20 laboratory-reared, late third-instar *A. aegypti* larvae. The mouths of 3 of these containers (chosen at random) were covered with gauze and acted as controls (adults were unable to enter and transfer JHA). The other 3 containers were open and accessible to ovipositing adults. No other food or moisture sources were made available. Either 500 or 50 female mosquitoes, blood-fed 1 day previously, were released into the cage (Fig. S1). After 5 days, all containers were collected and maintained until all larvae had died or emerged as adults. Any discrepancy between the final total and the 20 larvae originally placed in each tub was added to the mortality total (cadavers, exuviae, and weak individuals disappear as they are browsed upon by older instar larvae). In the open pots, the number of eggs oviposited and their subsequent eclosion was also monitored. The lining paper from each pot was collected and placed in a tray of tepid hay-infused water. After 3 days, papers were removed and all eggs were counted under a binocular microscope and scored as unhatched or hatched. For experiments involving the rolled sheet design, the number of eggs oviposited and the proportion of those eggs that were viable was also noted.

Statistical Analysis. For each caged experiment, mortality was compared between open and closed pots (treatments), after allowing for replicate differences, using logistic regression (generalized linear model with binomial error and logit link). To allow for overdispersion (indicated by a residual mean deviance significantly greater than unity) ratios of treatment to residual mean deviances were compared against the F-distribution. Approximate standard errors on the percentage scale were obtained by back-transformation from the logit scale. The percentage mortality in open pots (combining data from all runs) was regressed against the common logarithm of the number of eggs laid, again using logistic regression. For the 2 rolled sheet experiments the average proportion of non eclosed eggs in the open pots was estimated via a logistic regression incorporating only replicate effects.

Results: Caged Laboratory Experiments. Average mortality was greater in open pots than in closed pots for each combination of dissemination station type and number of females (rolled sheet with 500 females: $F_{1,14} = 49.58$, rolled sheet with 50 females: $F_{1,14} = 21.99$, pot design with 50 females: $F_{1,14} = 16.36$; all $P < 0.001$; Fig. S1). Clearly, the entry of contaminated adult mosquitoes to the larval microcosm resulted in contamination of the site and disruption of juvenile development.

The release of 500 blood-fed females into cages containing the rolled sheet resulted in an average 88% mortality (≈ SE = 5.2%) among the developing juvenile stages in open oviposition pots. In contrast the control pots (where gauze lids denied access to ovipositing females) exhibited 13% mortality (≈ SE = 5.3%). The release of 50 females per cage, with the same rolled sheet design, resulted in an average 9% (≈ SE = 5.6%) and 69% (≈ SE = 9.0%) mortality for the closed and open pots, respectively. When the trap design was changed to that of a pot, similar patterns emerged: 16% (≈ SE = 7.4%) and 71% (≈ SE = 9.0%) mortality for the closed and open containers, respectively. This served to show that both trap designs were similarly effective. The pot design was the simplest to deploy in our subsequent field experiments.

The mortality seen within the 3 open containers in any replicate could be highly variable (e.g., 0%, 60%, and 95% mortality using 50 females and the rolled sheet design; Fig. S2). That mortality was strongly correlated with the number of eggs laid (Fig. S2) for the rolled sheet (500 females: $F_{1,7} = 8.45$, $P = 0.023$; 50 females: $F_{1,7} = 12.28$, $P = 0.010$). Presumably, this reflects the number of visits that the containers received from ovipositing adults and therefore the quantity of JHA that was transferred. We can therefore infer that repeated contaminations of the aquatic habitat result in increasing concentrations of pyriproxyfen in the water and a concomitant increase in mortality among the juveniles developing therein.

All mosquitoes in these caged experiments had the opportunity to rest on the dissemination trap and oviposit in the open containers, so it was not possible to compare the viability of eggs laid by exposed females to eggs laid by unexposed females. However, of the eggs laid in the open pots (rolled sheet only), the vast majority did not hatch: 500 females, 79% failure (≈ SE = 0.9%); 50 females, 91% failure (≈ SE = 4.4%). This was expected from the results of our original studies (1). Pyriproxyfen exposure has profound effects on the fertility of female mosquitoes.

Simulation Model Assumptions, Definitions, and Limitations. The deterministic equation ($C_h = 1 - \exp(C_r U [O/H\Omega])$) considers that the availability of oviposition sites and larval habitats that remain productive in the presence of contaminated, ovipositing mosquitoes ($1 - C_h$) decreases exponentially with effective resting site coverage and the rate at which contaminated mos-

quitoes can render habitats unproductive. That rate, in simplistic terms, is the product of the rate of contaminated ovipositions by the total mosquito population (O) relative to the number of available aquatic habitats (H) divided by the mean number of contaminated ovipositions required to render a single habitat unproductive (Ω). Crucially, the proportion of habitats rendered unproductive at any time depends on the period over which contamination can suppress mosquito emergence (U). This latter parameter describes the effect of long-lasting insecticides on relatively stable habitats in which the impact of the insecticide is not being reduced through flushing effects or insecticide degradation. A summary of the model parameters is given in Table S1.

This simple model makes several assumptions:

(*i*) The distribution of oviposition events among development sites is random. Thus even 100% contamination of adults via resting sites will not yield 100% coverage of aquatic habitats ($O/H = 1$) unless the insecticidal activity lasts for many days. The reality is that contaminated mosquitoes are likely to target aquatic sites in a nonrandom manner, driven by the myriad cues that determine habitat suitability.

(*ii*) One gravid mosquito contaminates a single oviposition site. In fact, gravid *A. aegypti* practice "skip oviposition" and oviposit batches of eggs in a number of water bodies (2) but it is a reasonable assumption that adults can only carry enough insecticide to contaminate the first site visited. If this is incorrect, then it only serves to increase the impact of JHA transfer by increasing the contaminated proportion of O and raising oviposition site coverage (C_h).

(*iii*) Development sites contaminated by an oviposition event are not attractant or repellent to the mosquito. We have established previously that this is the case for pyriproxifen, the JHA used in our field demonstration (1).

(*iv*) We assume a single enclosed mosquito population. This is never the case in nature. Exchange of mosquitoes between neighboring subpopulations can have profound effects on the impacts of vector control tools (3) and, as vectors share and compete for resting and aquatic habitats with other species, it is possible that our method might be further optimized through contamination events mediated by other species. This might allow delivery through a wider variety of JHA targets, including nonhuman hosts, outdoor resting sites, and sugar sources used by nonvector mosquitoes.

The vast majority of vector population and vector-borne pathogen transmission models assume an enclosed system with single populations of mosquitoes, humans, and habitats, homogenously mixed and interacting at random. The absolute size of the ecosystem is irrelevant to simulated outcomes under these assumptions so we simply chose 1,000 larval habitats ($H = 1,000$) and tuned the overall oviposition rate (O) to give a range of values for the proportion of habitats that were oviposited in (O/H). The limitations of such simplistic models are well-described (3, 4) as is the real-world complexity of mosquito dispersal between hosts and oviposition sites in heterogeneous environments (2, 3). Our model should therefore be considered only as a simplistic exploration of our empirical findings within an otherwise complex field setting.

1. Sihuincha M, et al. (2005) Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Peru. *J Med Entomol* 42:620–630.
2. Reiter P (2007) Oviposition, dispersal, and survival in *Aedes aegypti*: Implications for the efficacy of control strategies. *Vector Borne Zoonotic Dis* 7:261–273.
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4. Gu WD, Regens JL, Beier JC, Novak RJ (2006) Source reduction of mosquito larval habitats has unexpected consequences on malaria transmission. *Proc Natl Acad Sci USA* 103:17560–17563.
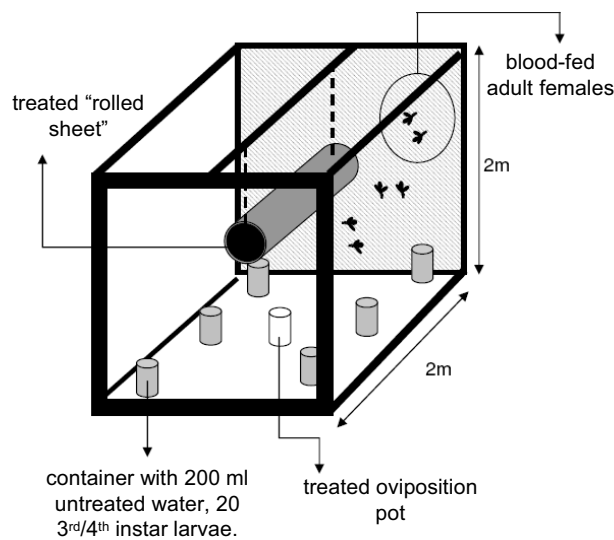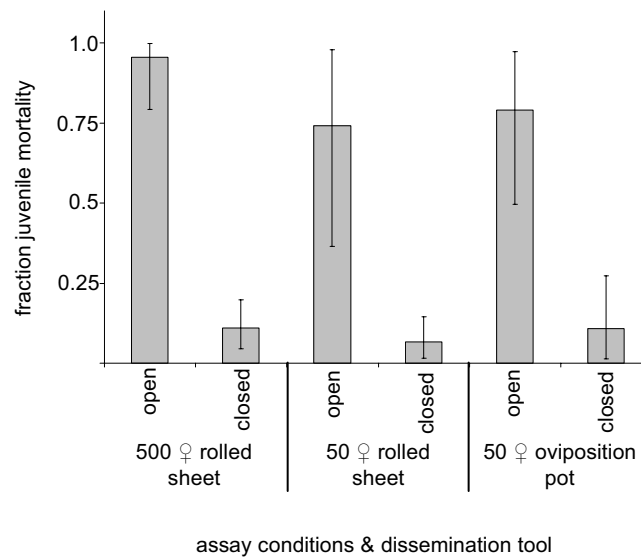
Fig. S1. Caged trials: mortality of juvenile stages developing in open pots (allowing access by contaminated adult female mosquitoes) and closed pots (adult mosquitoes prevented from entering) (mean ± 95% confidence limits). Schematic shows experimental design.

Detail from open
oviposition pot
showing thick band of
eggs and many dead
pupae
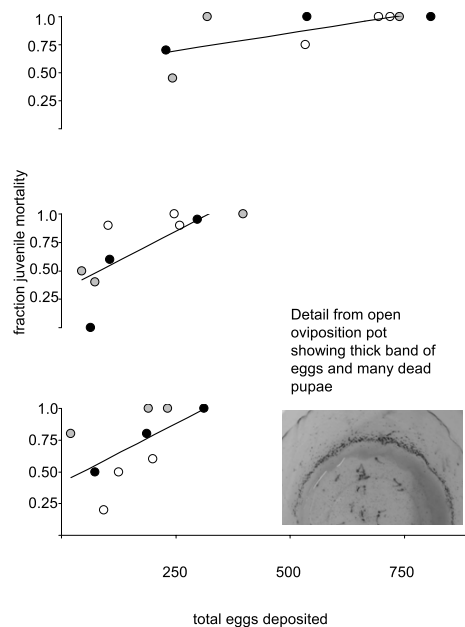
Fig. S2. Caged trials: relationship between mortality in cohorts of larvae developing in pots and number of eggs deposited in those pots (as a proxy for number of oviposition events). (*Top*) Rolled sheet design with 500 females . (*Middle*) rolled sheet design with 50 females. (*Bottom*) Oviposition pot design with 50 females. See *SI Text* for explanation. (*Inset*) Illustration of how large numbers of eggs, the result of multiple oviposition and contamination events, are associated with high pupal mortality.

Table S1. Summary and explanation of model parameters

Symbol	Definition	Unit
C_r	Effective insecticide coverage of resting site resources such as tombs, walls, bed nets, blood hosts, or sugar sources	Proportion
C_h	Effective coverage of larval habitats with sufficient levels of insecticide to prevent emergence of adult mosquitoes	Proportion
Ω	Number of contaminated ovipositions required to render a single habitat unproductive	Ovipositions per habitat
U	Interval for which habitat is unproductive after effective contamination (persistence of insecticide)	Days
O	Total contacts with an oviposition site by adult mosquitoes	Contacts per day
H	Number of aquatic habitats suitable for oviposition	Habitats